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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

Antonio J. GRILLO-LOPEZ et al. Group Art Unit: 1642

Application Serial No. 09/840,872 Examiner: Gary B. Nickol

Filed: April 25, 2001

Title: INTRATHECAL ADMINISTRATION OF RITUXIMAB FOR TREATMENT OF CENTRAL NERVOUS SYSTEM LYMPHOMAS

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DECLARATION BY ELLEN GARBER, PURSUANT TO 37 C.F.R. § 1.132

1. I am an Associate Director of Molecular Discovery at Biogen Idec Inc.

2. I direct the antibody engineering group in Biogen Idec's Cambridge Research facilities. I clone, chimerize, and humanize antibodies and clone, engineer, express, and optimize therapeutic proteins. I engineer expression vectors for therapeutic proteins, particularly for cell lines used in manufacture of recombinant protein drugs. I study receptor: ligand interactions and conduct structure/function studies on selected target genes.

3. I earned a PhD in Biology (major was in biochemistry and minor was in genetics) at Princeton University in 1979.

4. I have worked in the field of molecular biology for 25 years: two post-doctoral fellowships in molecular virology at The Rockefeller University (1979-1986), almost 6 years at Merck Research Laboratories (1986-1992) and over 12 years at legacy Biogen and Biogen Idec Inc (1993-present).

5. I have reviewed the journal publication by DeAngelis et al. (1998) *J. Neuro-Oncology* 38:245-252 and U.S. Patent No. 6,042,826 to Caliguiri et al.

6. I am also familiar with U.S. Patent No. 5,776,456 to Anderson et al.

7. I understand that the DeAngelis review article and the Caliguiri and Anderson patents were cited in an official action issued by the United States Patent and Trademark Office in connection with U.S. Patent Application No. 09/840,872 entitled "Intrathecal administration of rituximab for treatment of central nervous system lymphomas," and I have read the examiner's rejection of claims based on these documents.

8. I further understand that it is the examiner's position that the combined teachings of DeAngelis, Caligiuri, and Anderson, render obvious anti-CD20 therapy for the treatment of CNS lymphomas.

9. In particular, the examiner contends that "[w]hile the Caliguiri reference does not specifically teach administration of the claimed anti-CD20 antibodies, one of ordinary skill in the art who reads the Caliguiri reference would understand that the primary lymphomas of the CNS are treatable with antibodies – a lesson which is particularly relevant to the teachings of the Anderson patent which also concludes that lymphomas (in general) can be treated with antibodies." Advisory action, page 3, lines 16-20 (emphasis in original).

10. As described further below, I disagree with the examiner's analysis in that it focuses on the mechanics of intrathecal antibody administration without considering the biological basis of particular treatments.

11. With respect to the subject matter of the pending application, DeAngelis is a review article that states that intrathecal chemotherapy is a mainstay treatment for leptomeningeal metastasis and that this treatment is usually well-tolerated. *See* page 249, column 1.

12. As one of skill in the art, I disagree with the examiner's suggestion that the ongoing use of chemotherapy drugs by intrathecal administration, as summarized by DeAngelis, has predictive value regarding the therapeutic efficacy or safety of other drugs.

13. DeAngelis identifies three chemotherapeutic drugs that are commonly used for treatment of leptomeningeal metastasis: methotrexate, cytarabine, and thiopeta. *See* page 249, column 2. These are inorganic chemicals, which are completely different molecular entities than antibodies, which are biologic therapeutics.

14. The anti-cancer activity of classical chemotherapeutic drugs lies in their ability to disrupt basic cellular activities of actively dividing cells. For example, methotrexate and cytarabine are antimetabolites that interfere with nucleic acid synthesis by inhibiting folate metabolism.

15. In contrast, the therapeutic efficacy of targeting antibodies relies upon the cytotoxicity of associated molecules and/or cytotoxicity mediated by effector functions found in the antibody constant regions.

16. Notwithstanding the above-noted structural differences and distinct modes of action of chemotherapeutic drugs as compared to therapeutic antibodies, direct brain administration of any therapeutic agent remains highly unpredictable due to the unique brain environment and the risks of neurotoxicity.

17. I also strongly disagree with the examiner's conclusion that treatment of CNS lymphomas by intrathecal administration of anti-Fas antibodies, as described in the Caliguiri patent, renders obvious the potential therapeutic efficacy of anti-CD20 antibodies for treating CNS lymphomas.

18. The Caliguiri patent describes treatment of primary central nervous system lymphoma (PCNSL) using a Fas-cross-linking composition to elicit Fas-mediated cytotoxicity.

19. The Caliguiri patent states that the Fas-cross-linking composition can be an anti-Fas antibody having agonist activity or soluble Fas ligand.

20. Fas, also called APO-1 or CD95, is a transmembrane receptor that is a member of the tumor necrosis factor (TNF) receptor family, which trigger apoptosis by activating a cascade of specific proteases called caspases. The activated caspases cleave cellular components, a process that leads to morphological cellular and nuclear changes as well as to degradation of chromosomal DNA.

21. Following a review of the Caliguiri patent, I would not conclude that anti-CD20 antibodies could be reasonably predicted to have therapeutic benefit when administered intrathecally because anti-Fas antibodies and anti-CD20 antibodies recognize distinct antigens and confer therapeutic effects by entirely different modes of action.

22. In contrast to the agonistic anti-Fas antibodies described in Caliguiri, the therapeutic efficacy of anti-CD20 antibodies relies on induction of antibody-dependent cell-mediated cytotoxicity (ADCC) and cell dependent cytotoxicity (CDC).

23. ADCC refers to the destruction of target cells by natural killer (NK) cells when antibodies bound to the surface of a target cell interact with Fc receptors (FcR) on the NK cell.

24. Following a study of both mouse monoclonal antibodies and the humanized, clinically effective antibodies trastuzumab (HERCEPTIN®) and rituximab (RITUXAN®), a research group actively involved in the field of targeted immunotherapy concluded that "engagement of Fcγ receptors on effector cells is a dominant component of the *in vivo* activity of antibodies against tumors." *See* Clynes et al. (2000) *Nature Medicine* 6(4): 443-446, abstract (copy enclosed).

25. More recent studies have correlated responsiveness of patients with Waldenstroms Macrobulemia to rituximab therapy with a polymorphism in *FCGR3A*, the gene encoding FcγRIIIa subclass receptors expressed on both NK cells and macrophages. Specifically, the *FCGR3-158V* allotype displays a higher affinity for human IgG1 and increased ADCC. *See* Cartron et al. (2002) *Blood* 99(3): 754-758, abstract (copy enclosed)

26. A separate group has shown a similar relationship of FcγRIIIa genotype and the degree of B cell depletion by rituximab in patients with systemic lupus erythematosus. *See* Anolik et al. (2003) *Arthritis Rheum.* 48:455-459 (copy enclosed).

27. Elevated levels of circulating NK cells also correlate with patient responsiveness to the anti-CD20 antibody rituximab, suggesting that ADCC may be a primary mechanism by which rituximab functions. *See* Treon et al. (2005) *J. Clin. Oncol.* 23(3): 474-481, at page 475, column 1, 2nd paragraph (copy enclosed).

28. Collectively, the above-noted studies show that ADCC mediated by NK cells is a central component of anti-CD20 therapy.

29. Anti-CD20 antibodies can also elicit complement dependent cytotoxicity (CDC), which involves activation of the C1 complex by binding of the C1 complex component, C1q, to the Fc portions of antibodies in an immune complex. A sequence of enzymatic reactions ensues to generate C3 convertase, which in turn cleaves complement components that mark cells for destruction by macrophages.

30. Therefore, ADCC and CDC each require immune system effector cells, *i.e.*, NK cells and macrophages, to effect lysis of antibody-targeted cells.

31. Although the CNS participates in innate immune responses, including NK and macrophage activity, immune inhibitory and anti-inflammatory mechanisms physiologically

outbalance and counteract immune activity and thereby limit immune-mediated tissue damage in the brain. *See* Friese et al. (2004) *Onkologie* 27(5):487-491 (copy enclosed).

32. For example, under normal conditions and in the presence of intracranial tumors, levels of the complement proteins C3 and C4 are approximately 100-fold to 200-fold lower in cerebrospinal fluid (CSF) than in serum. In addition, complement activation and C3 antigen were undetectable in the CSF of cynomologous monkeys both before and after intrathecal administration of rituximab. *See* Rubenstein et al. (2003) *Blood* 101(2): 466-468, column 2 (copy enclosed).

33. Induction of apoptosis has been described as a third therapeutic mechanism of anti-CD20 antibodies. In order to elicit apoptosis of CD20-expressing cells, however, the presence of secondary IgG antibodies or FcR-expressing cells is additionally required. *See e.g.*, Shan et al. (1998) *Blood* 91(5)): 1644-1652 (copy enclosed) and Shan et al. (2000) *Cancer Immunol. Immunother.* 48(12): 673-683 (copy of abstract enclosed).

34. Therefore in contrast to the mechanism of action of classic chemotherapeutic drugs and anti-Fas antibodies, which directly induce apoptotic changes within tumor cells (*i.e.*, without the action of lymphoid and myeloid effector cells), the cytotoxicity of anti-CD20 antibodies depends on the recruitment of immune system effector cells, particularly FcR-expressing NK cells and macrophages.

35. The dependence on immune system effector cells, whose activity is limited in the CNS, is one reason why the therapeutic efficacy of anti-CD20 antibodies in the treatment of CNS lymphomas has been uncertain.

36. Therefore, at the time at which the instant application was filed, the use of anti-CD20 antibodies for the treatment of CNS lymphomas was unpredictable notwithstanding the success of intravenously administered rituximab for non-CNS lymphomas.

37. All statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9 November 2005
Date

Ellen Garber, PhD
Ellen Garber, PhD